Microbial Source Tracking

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US EPA Region 5

Genomics Training Workshop

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EPA/ORD Source Tracking Group

National Risk Management Research Laboratory
Water Supply & Water Resource Division
Microbial Contaminants Control Branch

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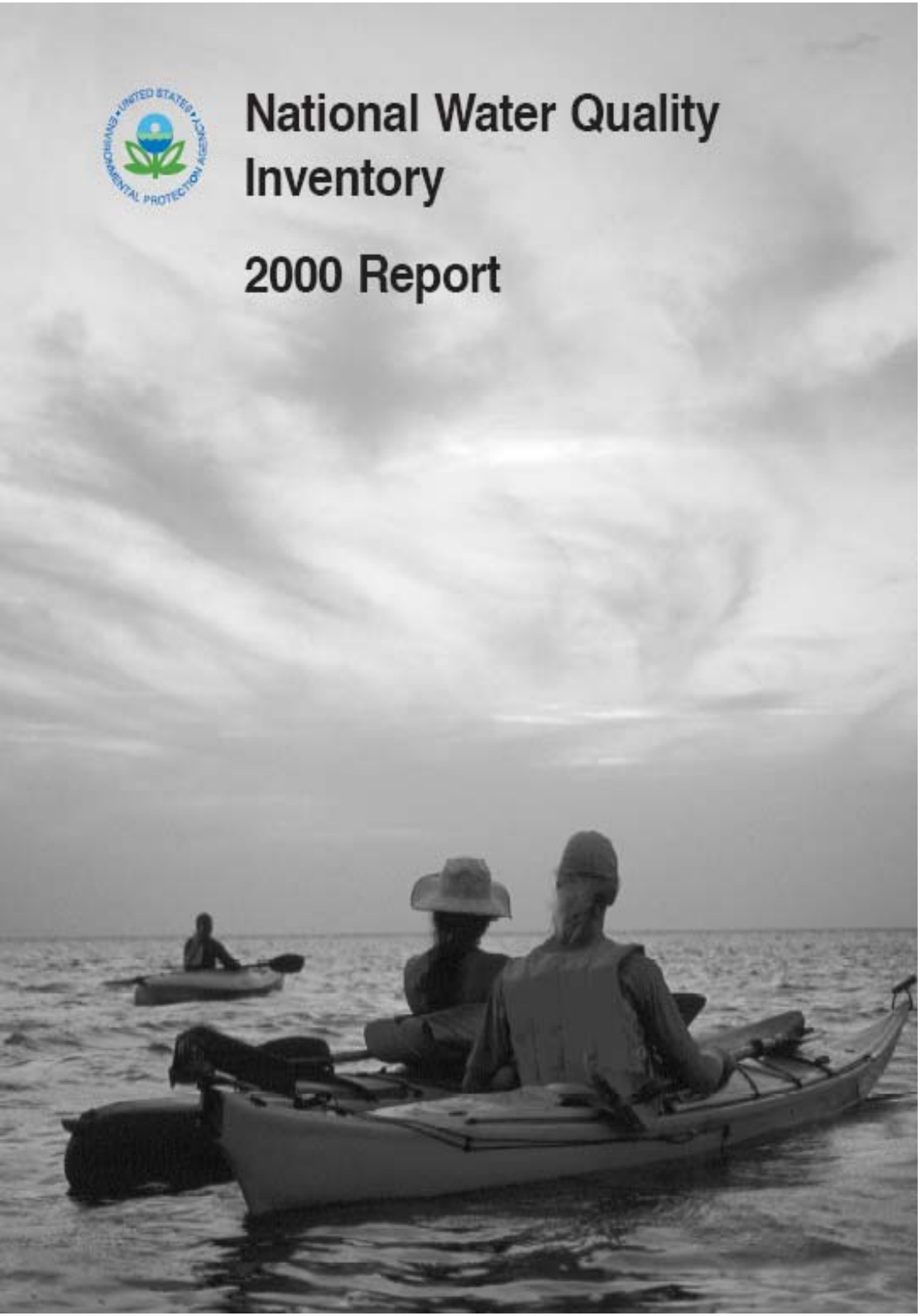
Microbial Source Tracking Presentation Outline

I. Overview

II. EPA Guide Document

III. Current Research



Sample Area:

39% rivers/streams (269K miles)

45% lakes/ponds (7.7 million acres)

51% estuaries (15K square miles)

Rivers and Streams:

Most common biological contaminant

13% bacterial pollution

35% of reported problems

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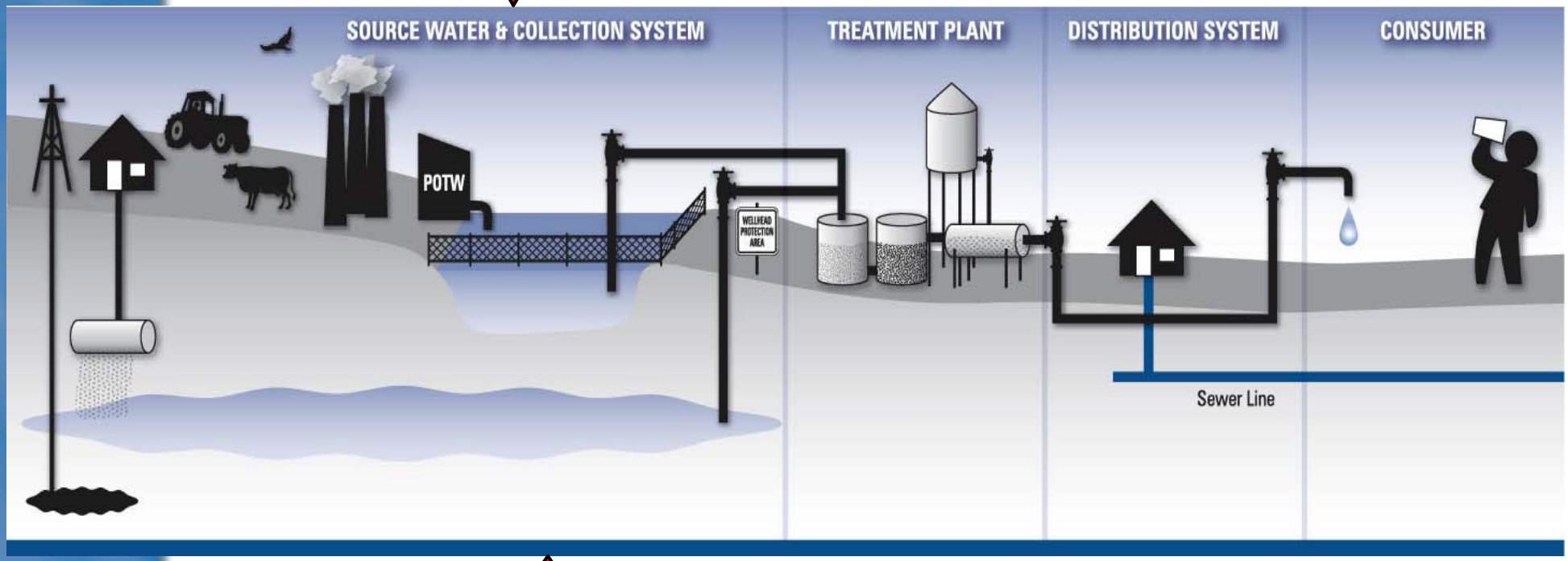
Protecting America's Public Health

PREVENTION with RISK ASSESSMENT

RISK



PREVENTION

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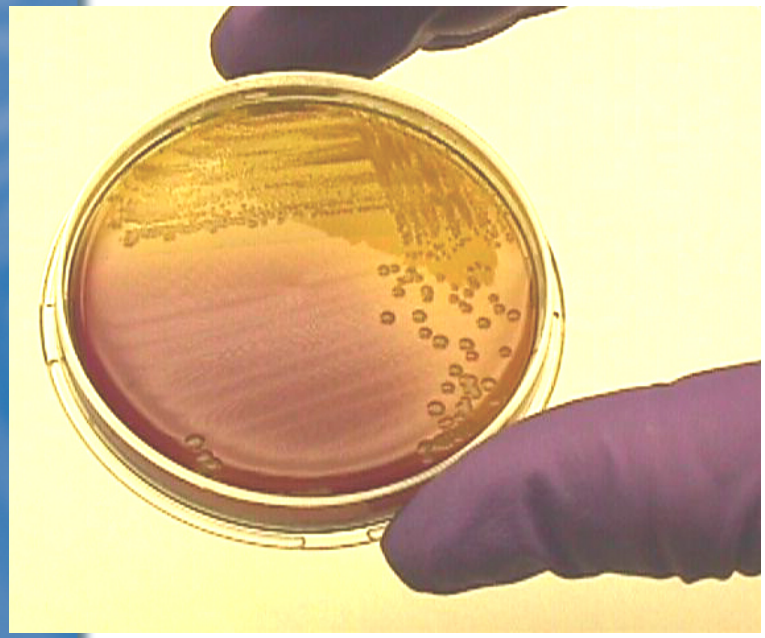
Monitoring Fecal Pollution

Microbial "Fecal Indicators"

- **Represents fecal pollution event**
- **Bacteria from animal intestine**



Traditional Methods

- **Presence/absence**
- **Count per unit volume**

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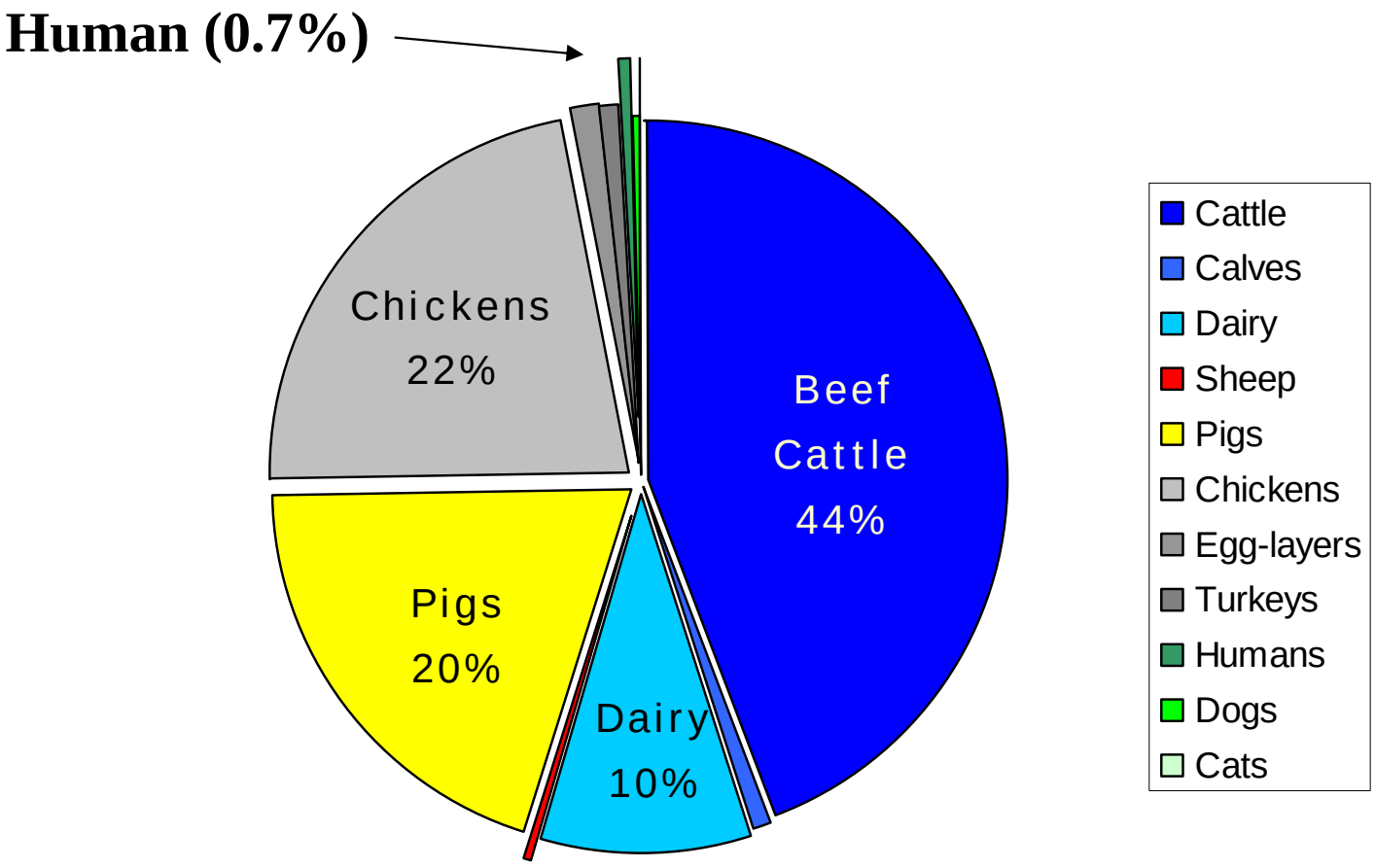
Feces Production in the U.S.



$1x10^{12}$ kg/year

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Wildlife Contributions

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Microbial Source Tracking

CONCEPT…. Match microbe from a polluted site and an animal source to suggest the origin of fecal pollution.

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When are Microbial Source Tracking Methods Useful?

To supplement sanitary surveys:

- Identify sources of beach contaminants
- Identify sources of TMDL violations

For risk analyses:

- Human versus non-human
- Human versus domestic animal

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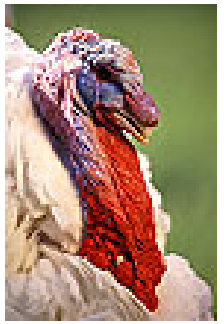
Why Should Microbial Source Tracking Work?

Intestinal microbes of animal groups are expected to be different:



- **Gut conditions**
  - **Temperature**
  - **Diet**
  - **Digestive system**

- **Natural selection**
  - **Space**
  - **Nutrients**

"Source Identifiers"

Definition . . . microbial populations that are particular to a specific animal host

Ideal Candidates:

- **Exhibit host-specificity**
- **Abundant in host**
- **Temporal stability**
- **Geographic continuity**

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Microbial Source Tracking Method Classifications

- **Qualitative vs. Quantitative**
- **Phenotypic vs. Genotypic**
- **Library-dependent vs. Library-independent**

Published:

Simpson, J. M., J. W. Santo Domingo, and D. J. Reasoner. 2002. Microbial Source Tracking – State of Science. Environ. Sci. & Tech. 36:5279-5288.

Library Dependent Methods

- **Library = "Fingerprint" database of *E. coli* or fecal enterococci isolates**
- **Requires 1,000s of isolates from water and suspected animal sources**
- **CULTURE-DEPENDENT**

Library Dependent Methods

- **ARA** (antibiotic resistance analysis)
- **CUP** (carbon utilization profiles)
- **PFGE** (pulse field gel electrophoresis)
- **RFLP** (restriction fragment length polymorphism)
- **AFLP** (amplified fragment length polymorphism)
- **RAPD** (random amplified polymorphic DNA)
- **rep-PCR** (repetitive extragenic palindromic)
- **Ribotyping** (RFLP using rDNA probes)

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Library Dependent Method Logistics

METHOD	Targets tested	Cultivation	Major Costs	Time Required*
ARA	•*Escherichia coli* •Fecal streptococci •*Enterococcus spp.*	•Individual •Isolates	•Antibiotics •96-well microplates	•4-5 days
CUP	•*Escherichia coli* •Fecal streptococci •*Enterococcus spp.*	•Individual •Isolates	•Microplates with substrates (e.g., Biolog, Phene Plate)	•2-5 days
rep-PCR	•*Escherichia coli*	•Individual •Isolates	•PCR reagents •PCR disposable •Gel electrophoresis	•1 day
RAPD	•*Escherichia coli*	•Individual •Isolates	•PCR reagents •PCR disposable •Gel electrophoresis reagents	•1 day
AFLP	•*Escherichia coli*	•Individual •Isolates	•DNA extraction kit •AFLP kit ($5 per reaction)	•5 days
PFGE	•*Escherichia coli* •*Enterococcus spp.*	•Individual •Isolates	•Plug prep. reagents •Restriction enzymes •Gel electrophoresis reagents	•2-4 days
Ribotyping	•*Escherichia coli* •Fecal streptococci •*Enterococcus* spp.	•Individual •Isolates	•DNA purification reagents •Gel electrophoresis reagents •Restriction enzymes •Hybridization/ detection solutions •Labeled gene probe	•1-3 days

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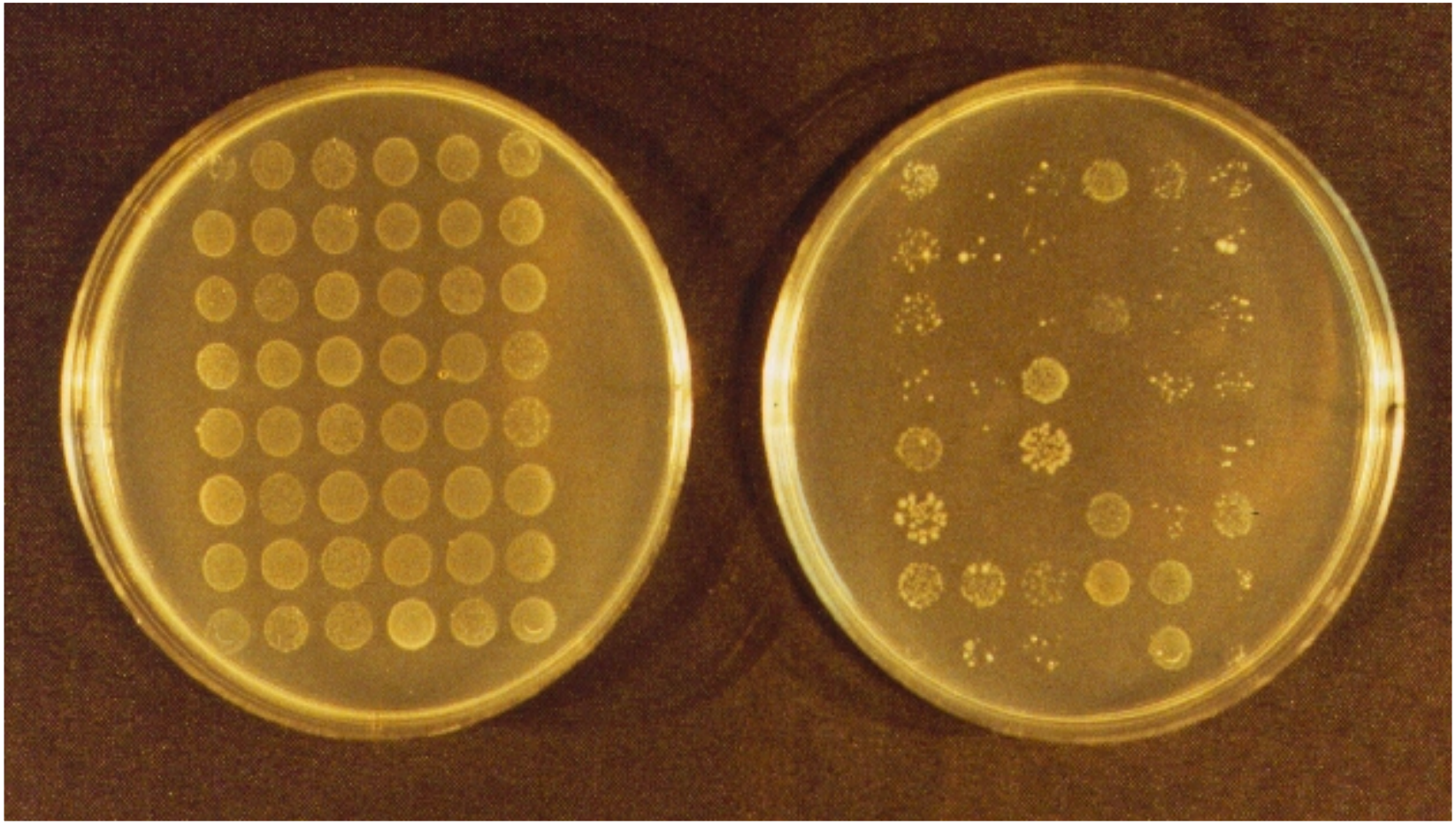
Library Dependent Method: Antibiotic Resistance Analysis



Advantages and Disadvantages of ARA

Advantages

- Easy to type
- Easy to perform
- Easy to interpret
- Inexpensive

Disadvantages

- Transferable trait
- Geographic specific
- Temporal specific
- Culture dependent
- Breaks down in complex watersheds

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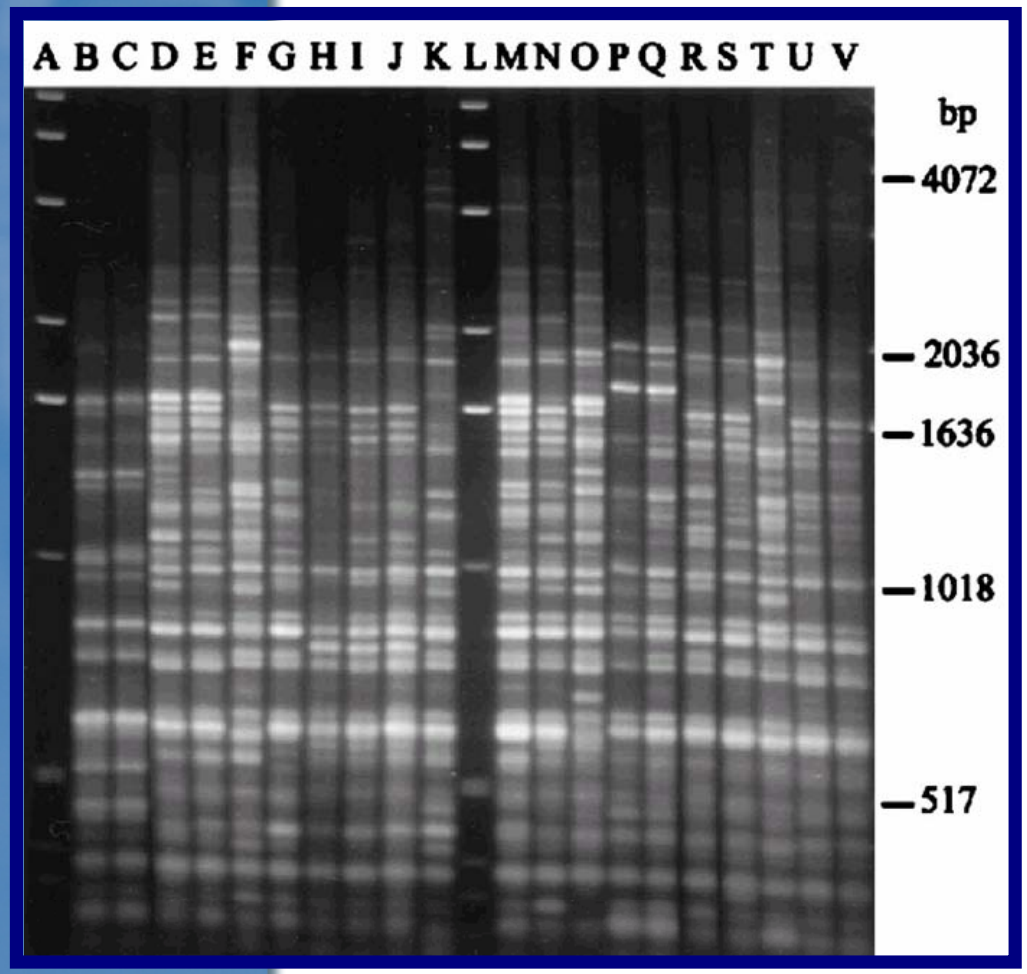
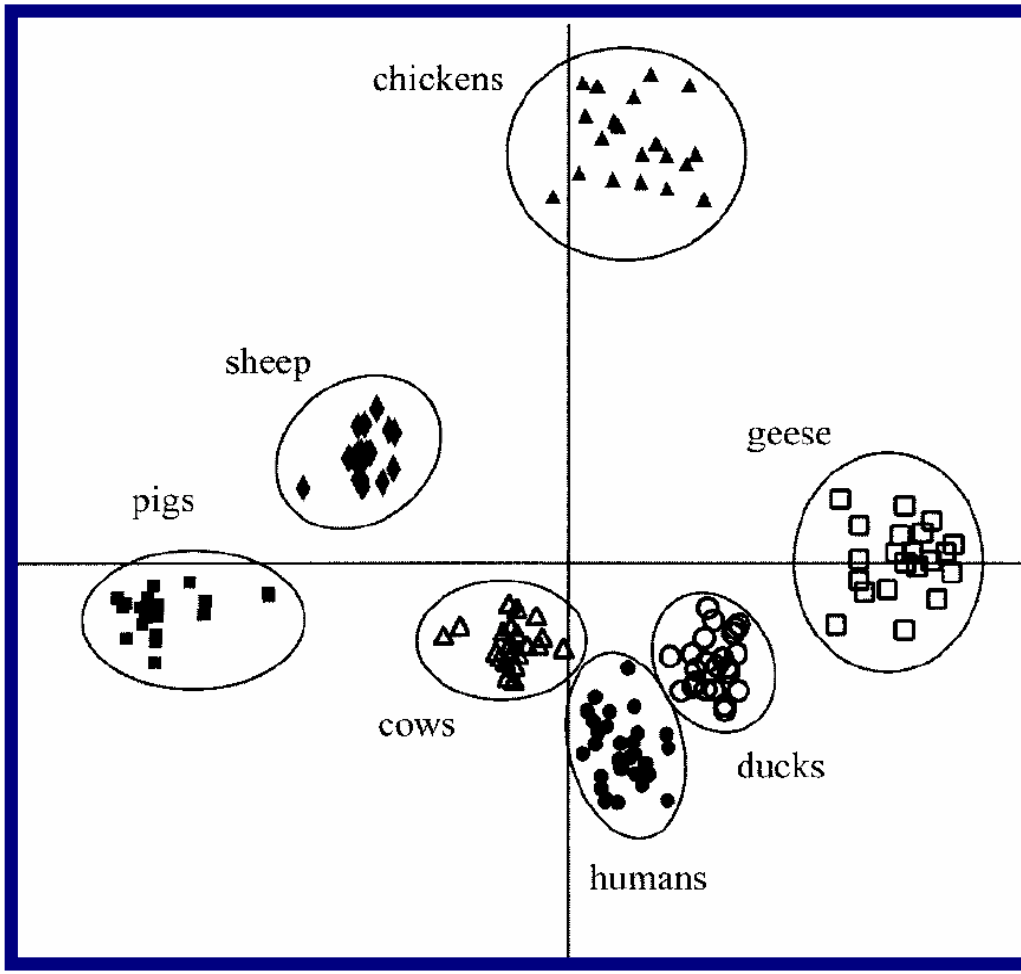
Library Dependent Method: rep-PCR DNA Fingerprint Patterns

(Dombek et al., 2000)

From this ...



... to this



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Advantages and Disadvantages of rep-PCR

Advantages

- Easy to type
- Easy to perform
- Easy to interpret
- Highly reproducible

Disadvantages

- Library dependent
- May be geographic specific
- May be temporal specific
- Culture dependent

Library Independent Methods

- **Phage typing (serotypic or genotypic)**
- **Gene specific PCR**
- **Total Community Analysis**
- **Host-specific PCR**

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Library Independent Method Logistics

METHOD	Targets tested	Cultivation	Major Costs	Time Required*
Phage Typing	• F+ coliphage	•Individual •Isolates	•Hybridization/ detection solutions •Labeled gene probe •Phage specific antigen	•1-3 days
Gene Specific PCR	• *E. coli* toxins	•Sample Enrichment	•PCR reagents •PCR disposables	•2 days
Total Community Analysis	• 16S rRNA	**•None**	•Filtration units •PCR reagents •PCR disposables •DNA sequencing	•1 month
Host Specific PCR	• *Bacteroides* • *Bifidobacteria* • *Enterococcus* • *Rhodococcus* • *F+ coliphage* • *Enterovirus* • *Adenovirus*	**•None**	•Filtration units •PCR reagents •PCR disposable	•6-8 hours

Host-Specific PCR: Bacteroides 16S rDNA

Bernard & Field, (2000) AEM *66, 4571-4574*
Dick et al, (2005) AEM *in press*

- **Primer sets that discriminate between human, ruminant, horse, and pig fecal pollution**
- **Target 16S rDNA from fecal *Bacteroides***
- **Successful in fresh and marine waters**

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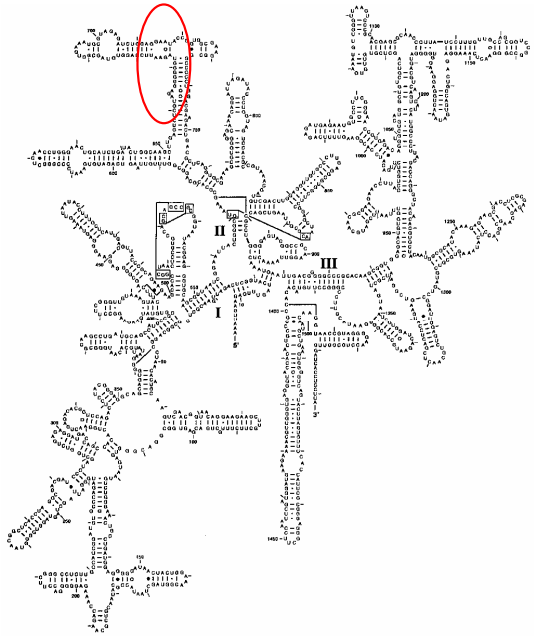
Why *Bacteroides* as a Source Identifier?

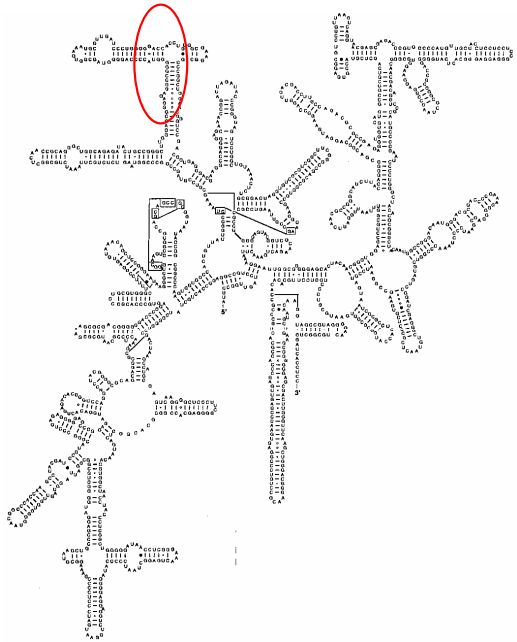
- **Only found in feces, rumen, and body cavities**
- **1/3 of fecal flora**
- **Obligate anaerobes**
- **Limited survival in environment**
- **Host-specific variation in animal hosts**

Comparative Sequence Analysis of 16S rRNA

16S rRNA of *Bacteroides mooii*



16S rRNA of *Bacteroides horseii*



	650	660	670
B. mooii	GCUUGAGUCU	CGUAGAGGGG	GGUAGAAUUC
B. horseii	GCUAGAGUAU	GGGAGAGGAU	GGUAGAAUUC

Advantages of Host-Specific PCR

- Culture independent
- No library required
- Rapid
- Sensitive
- Defined target
- Isolate target in a complex environment
- Automated analysis

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Current Limitations of Host-Specific PCR

- PCR inhibition
- Targets only one gene
- Targets only one bacterial group
- Targets are found in low numbers
- Limited number of case studies
- Small target sequence databases
- Current targeted genes have little to do with host/microbe interactions

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PART II

US EPA Microbial Source Tracking Guide Document

Office of Research and Development

National Risk Management Research Laboratory

Water Supply & Water Resource Division

Microbial Contaminants Control Branch

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Why is a Guide Document Needed?

Recent proliferation of new methods

- Genotypic
- Phenotypic
- Culture-based
- Culture-independent
- Different levels of discrimination

Most useful method depends on circumstances

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Content of Microbial Source Tracking Guide Document

I. Introduction

- What is Microbial Source Tracking?
- Definitions of terms

II. Decision Criteria

- When methods should be used
- Importance of sanitary surveys
- Decision tree

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Decision Tree

Questions:

- Is the problem adequately defined?
- Has an adequate sanitary survey been conducted?
- How many sources were identified?
- Is the study area of manageable size?
- What is the desired level discrimination?

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Content of Microbial Source Tracking Guide Document

III. MST Approaches

- Summary of all current methods
  - *Explanations of how they work*
  - *Summary tables with advantages and disadvantages*
  - *References*

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Content of Microbial Source Tracking Guide Document

IV. Data Collection and Analysis

- Design sampling around study objectives
- General principles for sampling
- Library construction and validation
- Spatial and temporal variability
- Similarity measurement methods

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Content of Microbial Source Tracking Guide Document

V. Performance Standards

- Universal quality measures
- Method-specific controls
- Method-specific performance criteria

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Content of Microbial Source Tracking Guide Document

VI. Assumptions and Limitations

- Characteristics of source identifiers
  - Specificity
  - Distribution in host
  - Geographic range
  - Temporal stability
  - Survival in water

Content of Microbial Source Tracking Guide Document

VII. Applications of Microbial Source Tracking Approaches

- Eight case studies are presented
- A glossary of terms is presented

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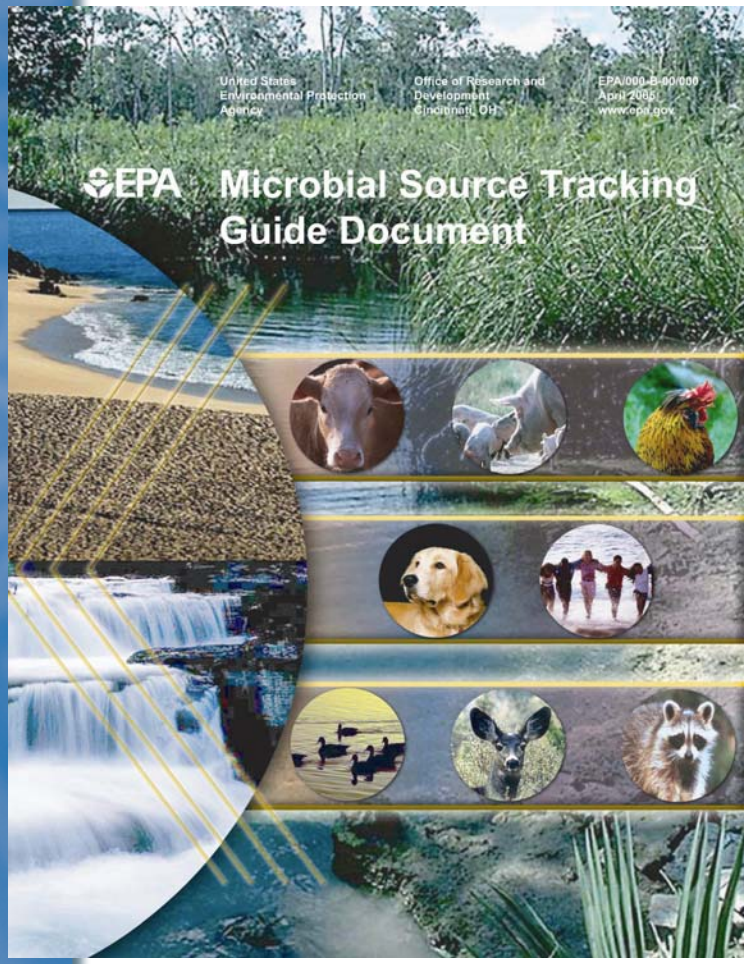
The Guide Document is Now Available!



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PART III

Current Research

EPA/ORD Source Tracking Group

Office of Research and Development
National Risk Management Research Laboratory
Water Supply & Water Resource Division
Microbial Contaminants Control Branch

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Current Research

- **Expand Library of 16S rDNA Sequences from Fecal Sources**
- **Validation of *Bacteroides* 16S rDNA Host-Specific PCR Method**
- **Evaluation of Best Management Practices**
- **Discovery of Novel Source Identifiers**

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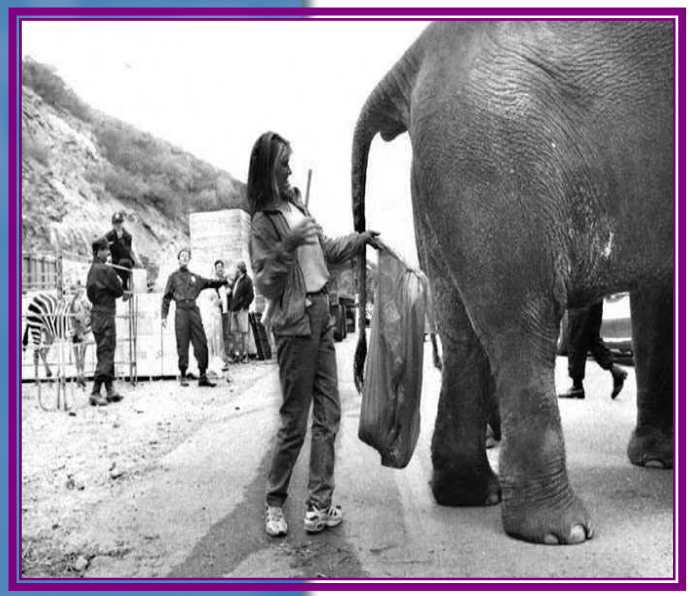
USEPA 16S rDNA Sequence Fecal Microbe Library

- **Fecal Sources (n = 300)**
  - **Domestic animals**
  - **Wildlife**
  - **Humans**



- **16S rDNA sequences**
  - **Bacteroidales (n = 1,000)**
  - **Clostridium (n = 500)**
  - **Enterococci (n = 1,500)**
  - **Bifidobacterium (n = 100)**

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Host-Specific 16S rDNA PCR Method Validation: Target Specificity

- **Test host-specific primer sets against fecal library**
  - **Ruminant-specific**
  - **Human-specific**
  - **Pig-specific**
  - **Horse-specific**
- **If cross-specificity observed, then**
  - **Sequence 16S rRNA**
  - **Add to database**

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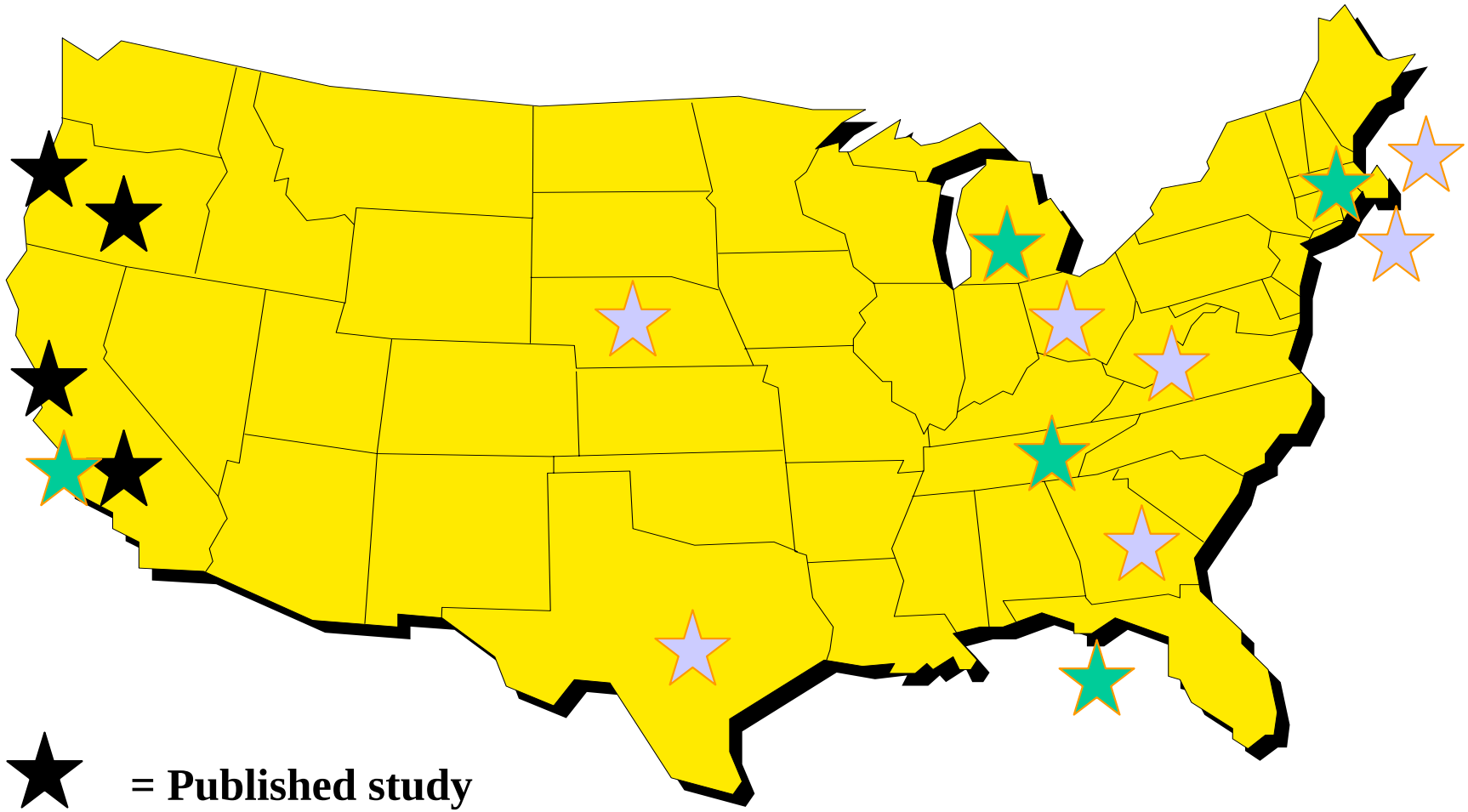
Host-Specific 16S rDNA PCR Method Validation: Spatial Stability



= Published study

= EPA

= Others

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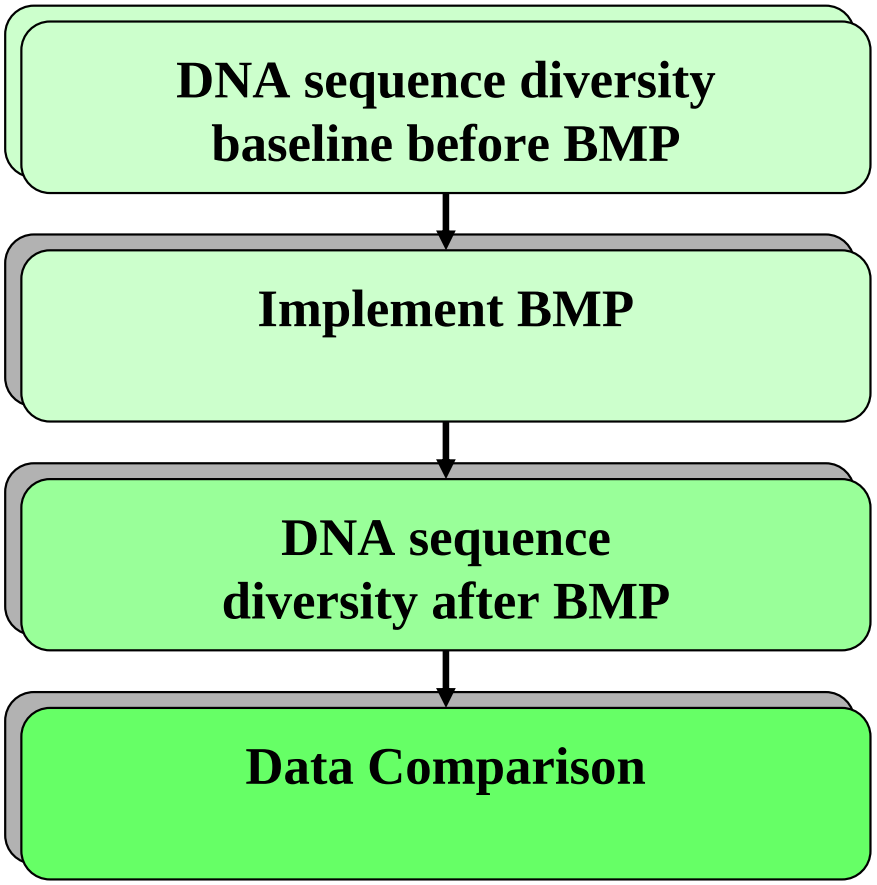
Delaware Project: Best Management Practice Evaluation

(Collaboration with DelawareDepartment of Natural Resources and Environmental Control)

Method Overview



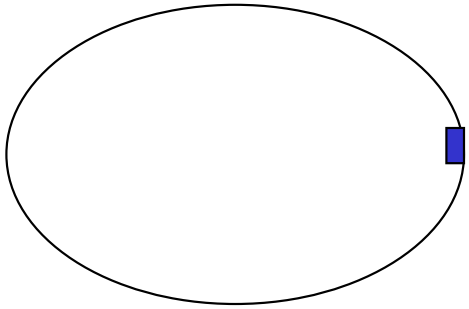
Progress

- Cows, sediment, & water
- 700 16S rRNA sequences

- Fences installed (Spring 2004)

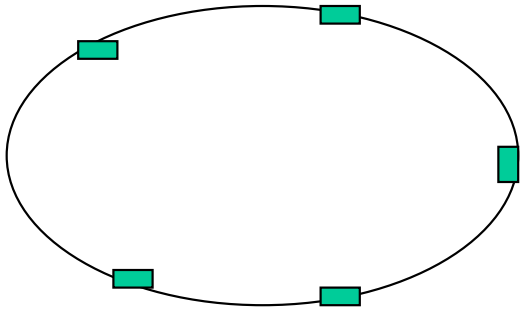
- Fecal, sediment, & water collection (Current)

Discovery of Novel Source Identifiers



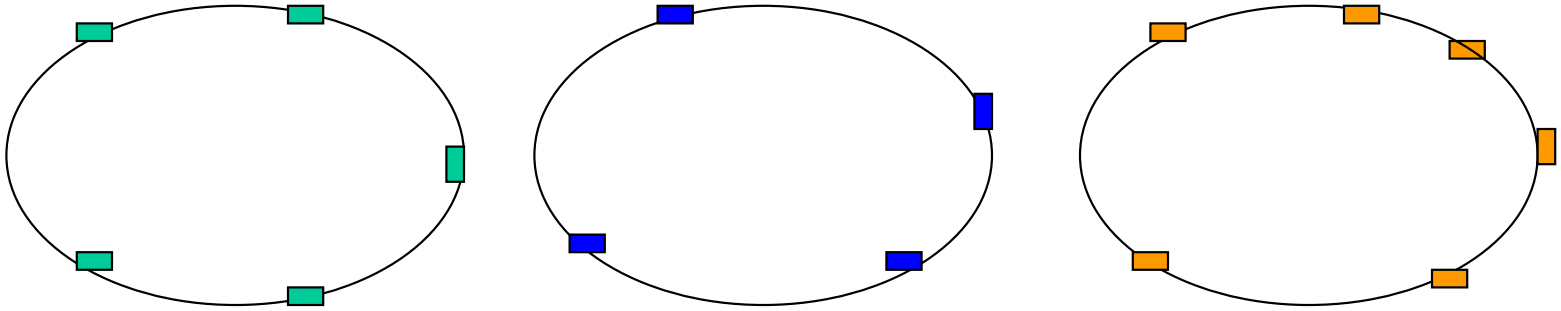
One gene – one group

PHYLOGENETIC Approach



Multiple genes – one bacterial group

GENOMICS Approach



Multiple genes – Multiple bacterial groups

METAGENOMICS Approach

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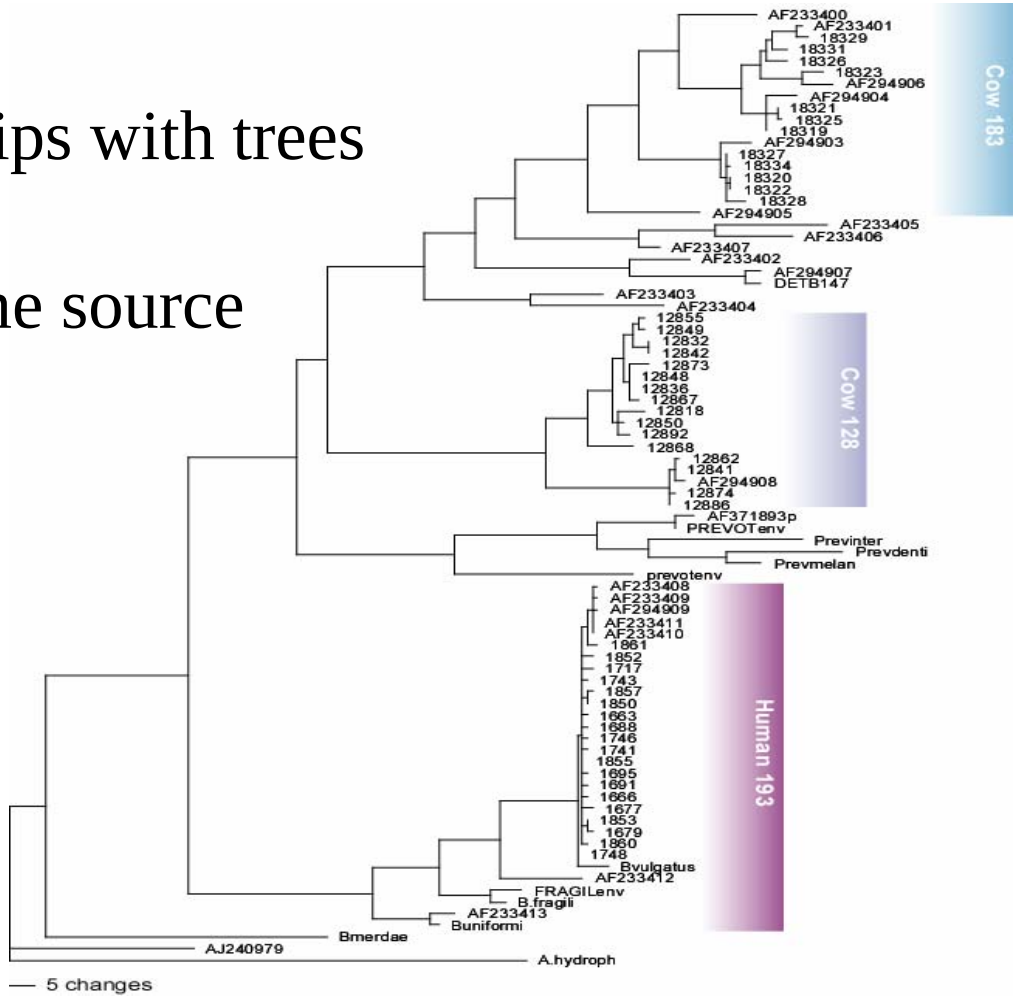
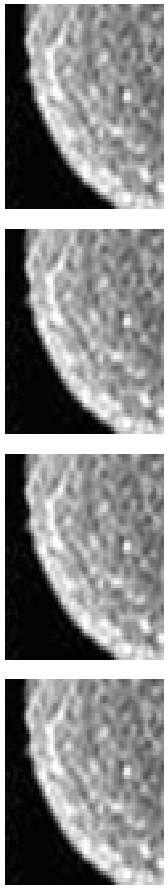
Use of Phylogenetics to Design New Host-Specific 16S rDNA PCR Assays

- Survey evolutionary relationship between *Bacteroides* from different sources
- Visualize relationships with trees
- Sequences from same source can cluster together
- Approach led to a horse-specific PCR assay

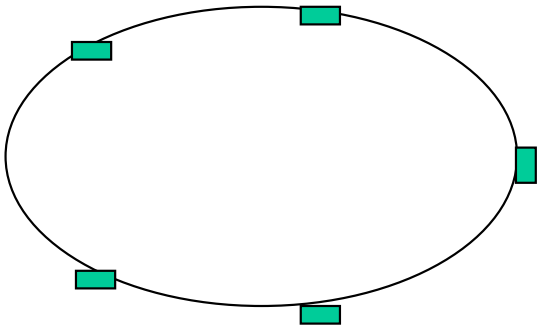
  (in press, AEM June 2005)



Use of Genomics to Identify Species-Specific DNA Sequences



- Standard for measuring fecal pollution
- Opportunistic pathogens
- Enterococci genomes already sequenced *(E. faecalis and E. faecium)*



Multiple genes – one bacterial group

GENOMICS

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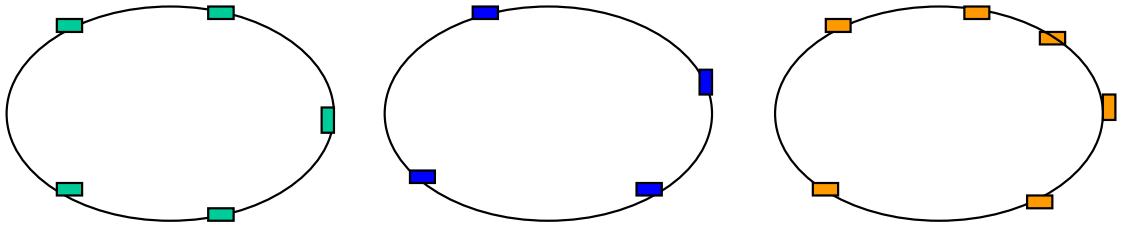
Use of Metagenomics to Design Cow-Specific PCR Assays

- Comparison of *Bos taurus* and *Sus scrofa* genome communities
- Access to yet to be cultured microorganism genomes
- Identification of non-16S rRNA host-specific DNA targets



Multiple genes – Multiple bacterial groups

METAGENOMICS

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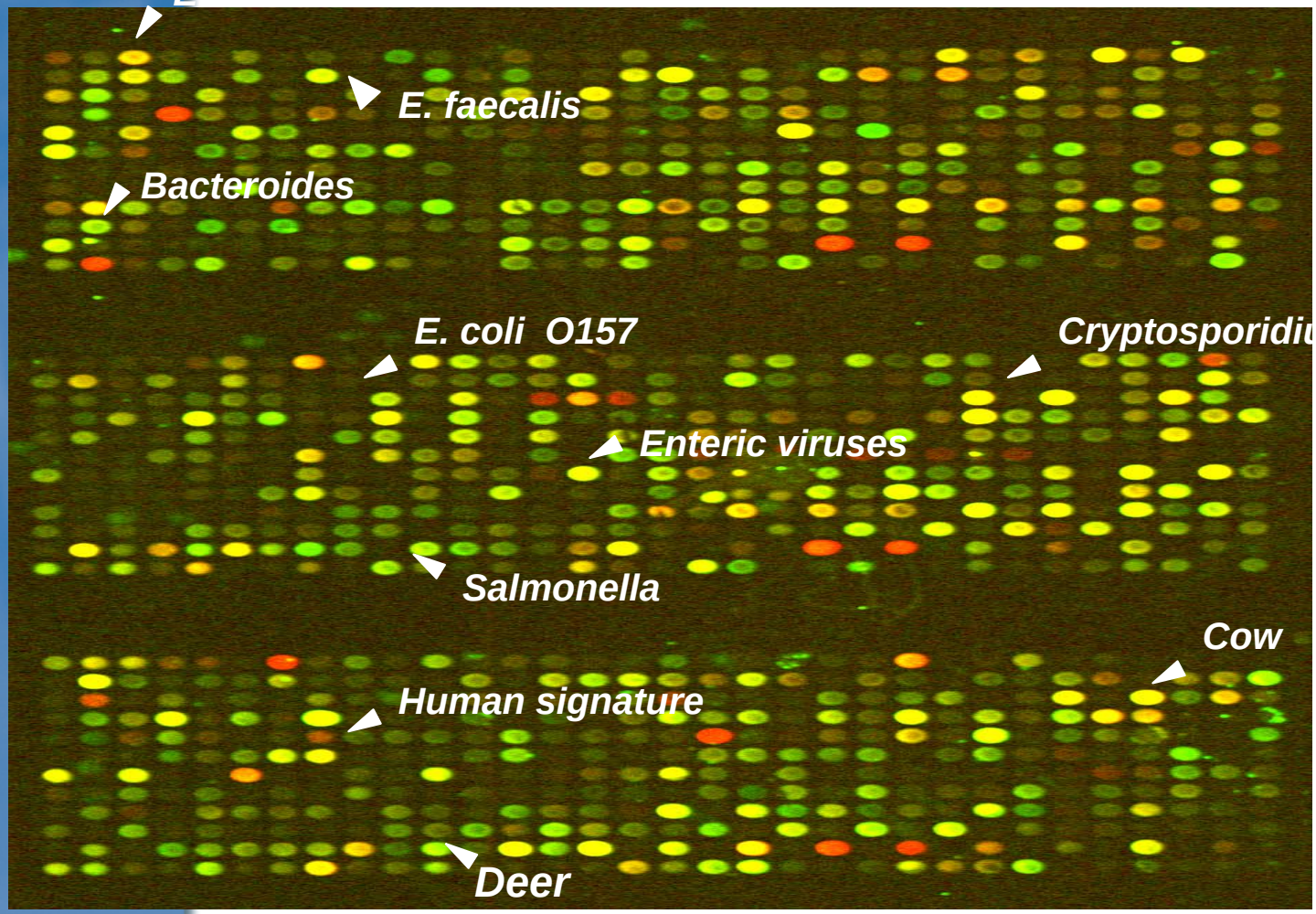
The Future of Source Tracking

- New methods will arise
- Some methods will become obsolete



Indicators

Pathogens

MST

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USEPA ORD Support for Microbial Source Tracking

- Guide Document
- Regional Workshops
- Collaboration
- Development of Regional Centers of Excellence